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13. ABSTRACT ***(Maximum 200 Words)***

Humans and most other organisms manifest circadian (daily) rhythms that are controlled by an endogenous biochemical oscillator. These "biological clocks" are important to human physiology. For example, psychiatric and medical studies have shown that circadian rhythmicity is involved in some forms of depressive illness, "jet lag," drug tolerance/efficacy, memory, and insomnia. Therefore, understanding the biochemical mechanism of circadian clocks may lead to procedures which will be useful in the diagnosis and treatment of disorders that are relevant to sleep, mental health, and pharmacology. Although recent breakthroughs in the field of circadian rhythms have identified a number of proteins that appear to act as clock components, we have only just begun to understand how these components interact functionally with themselves and the environment to generate a highly precise 24 hour oscillation that is temperature compensated and entrained to the daily cycle. We will test hypotheses concerning the significance of rhythmic clock protein abundance by using new methods to introduce proteins directly into cells by peptide-mediated transduction across cell membranes. These studies will yield results of theoretical importance, but also have the potential for designing treatments for jet lag, insomnia, and other clock-related disorders.

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Final Performance Report: "Cell-permeable Circadian Clock Proteins"

Significant progress was made during the brief tenure of this award, especially in view of the fact that problems associated with bringing a postdoctoral fellow to perform the work considerably delayed the effective starting date of the project. That is, the project start date was July 1, 2001, but the postdoctoral fellow could not arrive until early December, 2001. Several events transpired to create the delay. When it became clear that the AFOSR would fund the project, advertising and recruiting for an experienced postdoctoral fellow began. Fortunately, an excellent candidate was found in the person of Dr. Yunzhen Fan in early June. Dr. Fan had prior experience with making cell-permeant proteins in China (proteins related to apoptosis), and she was the only applicant from approximately 50 applicants with that crucial experience. An offer was made to her, which she readily accepted. Unfortunately, she did not have a passport, so she applied for a passport to the Chinese government, receiving it a month later. We then initiated the process of obtaining an appropriate visa for her. In the past, obtaining J-1 visas for postdoctoral candidates from China was routine, and therefore the J-1 (IAP-66) process was initiated. Dr. Fan made an appointment in late July with the U.S.A. Embassy in China. Surprisingly, her application for a J-1 visa was denied on the basis that she could not prove that she would return to China at the end of the postdoctoral fellowship. I appealed the case to the U.S.A. Embassy both directly and through the office of our Tennessee Senator, the Honorable William Frist, but both appeals generated the same negative response from the U.S.A. Embassy. I then consulted with the International Office at Vanderbilt University and was told that such refusals to grant J-1 visas to Chinese scientists had become common. I was advised to apply for an H-1 visa for Dr. Fan because the refusal rate was much lower for H-1 visas than for J-1 visas. However, the process for granting an H-1 visa normally takes 3-4 months, which was an unacceptable delay for our grant. I was therefore advised to pay an extra $ 1000 for expedited H-1 consideration. I therefore used discretionary (non-grant) funds to pay this additional $ 1000 fee to expedite the paperwork for Dr. Fan's H-1 visa. This process was completed in late August, and the paperwork was sent by Federal Express to Dr. Fan. She made another appointment with the Embassy in China. Unfortunately, the September 11th attack on the WTC towers and the Pentagon caused further delays in the consideration of her application. For example, with heightened security measures in place after the 9/11 attack and the anthrax mailings, her application received additional scrutiny when it was noticed that she had done some experiments with plant viruses. Apparently the dutiful bureaucrat who noted this fact did not realize that plant viruses are highly host specific and totally nonpathogenic to humans. Dr. Fan's application was therefore forwarded from the Embassy in China to the State Department in Washington, D.C. Consequently, I sent FAXes to both the Embassy in China and to the State Department. Finally, Dr. Fan's H-1 visa application was finally and officially approved in late October. Dr. Fan then informed me that she had family arrangements to make that would require about five weeks and that she would be able to arrive in early December. Once she arrived, there were numerous delays relating to obtaining a Social Security Card due to her arrival so close to the Christmas season, but that is another story that is not worth describing.

Once Dr. Fan finally arrived in the laboratory, she fulfilled my expectations of her that were based on her application. She is an excellent molecular geneticist with exceptional talents in the expression and purification of recombinant proteins. She has remade our original cell-permeant mCry2 construct with a more efficient transduction moiety that includes a nuclear

translocation motif. She has demonstrated that this new construct can be used to generate cell-permeant proteins that transduce into cells in culture and repress the mPer1 promoter. She is now in the position to use this new protein to test the models described in our original proposal—the only thing lacking at this point is the generation of an appropriate cell-permeant control protein, and she is currently working hard on that goal. She has also remade several of her constructs for expression in the baculovirus protein expression system. All our work to date has used bacteria (*E.coli*) for the expression of the cell permeant proteins. However, it might be that expression in the eukaryotic baculoviral system will be superior because there may be eukaryotic protein modifications to the cell-permeant proteins that will occur properly in the baculoviral system that cannot occur in the bacterial system. Therefore, Dr. Fan will compare the efficacy of cell-permeant clock proteins expressed from the eukaryotic system with those expressed from the bacterial system.

Because of the delayed effective start date of the grant due to the difficulty of getting visa approval for Dr. Fan, there were residual funds for which approval was granted for the purchase of two items of equipment (a plate-reading luminometer and a French Pressure cell) that will significantly aid the further experiments of this project. The luminometer will be used for the transfection assays that are our method of monitoring the efficacy of the cell-permeable proteins.

Preliminary data from this project were used in a grant proposal to the NIH (the R21 mechanism). This proposal was successful, and will provide for two more years of support for the project. If future publications result, the support from the AFOSR will be acknowledged as this award was crucial for the maintenance of the research.